Rejection of Claims 1-6 under 35 U.S.C.§112, First Paragraph

Claims 1-6 were rejected in the Action mailed October 1, 2003 as lacking enablement because the subject matter purportedly was not described sufficiently to enable one skilled in the art to which it pertains to make and use the invention. The Action takes the position that the claims are unduly broad and lack working examples. Additionally, the Action maintains that recombinase-mediated excision of flanked DNA sequences is unpredictable and thus would require excessive experimentation.

Attachments A and B provide supporting data

The Examiner's attention is directed to Applicant's Declaration in Exhibit A and to the working examples described in detail in Attachments A and B. The data clearly show that Applicants' examples in the instant application, in combination with the detailed description, are fully enabling.

Additionally, applicants have presented a new set of claims fully supported in the specification and by the working examples.

Rejection of Claims1-6 under 35 U.S.C. §102

Claims 1-6 remain rejected under 35 U.S.C.§102(b) as anticipated by the Oliver, *et al.* patent, US Patent No. 5,723,765. The Action generalizes the patent as teaching a cassette with all the elements claimed in Applicants' cassette. In particular, the Oliver patent is said to contain at least one pair of DNA excision sequences that flank heterologous DNA, the sequences being cleavable by a recombinase protein. As subsequently discussed, the Oliver constructs are not only different from the claimed cassettes, but also produce a different result.

With respect to the anticipation as well as to the enablement rejections under §112, first paragraph, Applicants direct Examiner's attention to new claims 25-48. The claimed cassettes are distinguishable from the gene cassettes employed by Oliver, *et al.* as well illustrated in the examples provided in Applicants' declaration. The data presented with Applicants' Declaration, support and confirm the examples and description in the instant application. Particular examples of the cassettes are set forth in the drawings, illustrated schematically in Figs. 1A,B, 2A,B and 3A,B (Attachment A). Data are provided showing that virtually the entire heterologous DNA introduced into the plant is excised.

Oliver, *et al.* utilize a different cassette from the disclosed cassettes, which are designed for a different purpose and, importantly, to achieve different results. Oliver, *et al.* link a

transiently active promoter to a structural gene where the structural gene and a promoter are separated by a blocking gene. The blocking gene is flanked on each side by an excision sequence, and a repressible promoter operably linked to a gene encoding a site-specific recombinase that recognizes the excision sequence. The DNA sequence also includes a gene encoding a repressor specific for the repressible promoter sensitive to an external stimulus. The structural gene is not expressed if the external stimulus is not present. When stimulated, the repressor function is inhibited, and the recombinase is expressed, causing removal of the blocking sequence at the excision sites. Once the blocking gene is removed, the structural gene is operated on by the promoter and is expressed.

Oliver, *et al.*, while employing a gene cassette containing the elements of a pair of cleavable DNA excision sequences that flank heterologous DNA, utilizes a different cassette and does not remove the structural gene of interest.

The Invention

Applicants have pointed out an important problem to be solved with respect to agricultural use of transgenic plants. Of particular concern is the recombination that may manifest in seeds or fruits of plants. During an earlier stage of development, these transgenic plants may have obtained benefit from an expressed transgene product that discourages or kills larvae of pests attacking the immature plant but is not important in pollen, seed, or fruit or at later stages of the mature plant and which in fact may be of concern in food or feed products.

Applicants have addressed this problem by engineering heterologous DNA cassettes that can be excised from a transformed plant after selected promoters are activated in specific organs, or by developmental events or by external stimuli. Remarkably, excision of the cassette is virtually 100% complete with no evidence of recombination when both LoxP and FRT recognition sequences are used. Only trace amounts of heterologous DNA (short and non-functional DNA fragment) from the recombinase or excision sites are left in the host genome while the genes of interest in the originally transformed plant are completely excised. The original transgenic plant, while technically still "transgenic" has a genome virtually identical to its wild-type genome.

Novelty and Non-obviousness

State of the Art

Oliver, *et al.* were concerned with excising a sequence that blocked a particular transgene. They engineered a cassette harboring a gene of interest separated from its linked promoter by a blocking sequence that in turn was flanked by an excision sequence. A recombinase DNA was also present in the cassette, which when activated by an inducible promoter, expressed a protein that binds to the excision sequences and excises the blocking sequence. The gene of interest could then be expressed in plants in which the cassette was incorporated.

Oliver, *et al.* fail to disclose or even suggest a method that effectively and completely removes functional transgenes from a transgenic plant. Instead, Oliver, et al. remove a blocking sequence that allows the gene of interest to be expressed. The gene of interest is not excised. In contrast, Applicants excise not only the gene of interest but also the vast majority of introduced heterologous DNA, thus preventing expression of the plant transgene responsible for the disclosed phenotypic trait. Once the recombinase is expressed, only an inconsequential amount of DNA from the flanking sequences remains, presumed to result from recombination.

New Claims

Applicants submit that the new claims fully address the issues previously raised by the examiner. The claimed subject matter is supported throughout the specification and is further confirmed with the supporting data presented in Attachments A and B and by Applicants' declaration (Exhibit A).

It is believed that the claims are in condition for allowance and reconsideration is respectfully requested. Should the Examiner have any questions, comments or suggestions, the undersigned requests a telephone conference at the number provided.

Respectfully submitted,

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CLAIMS

Claims 1-24 (canceled).

25. (new) A method for reversible introduction of heterologous DNA into a plant genome, comprising:

introducing a gene cassette into a plant, said gene cassette comprising a heterologous DNA conferring a trait of interest, said heterologous DNA flanked 3' by at least a first DNA recombinase recognition sequence and flanked 5' by at least one DNA encoding a first recombinase operably linked to an organ-specific, developmental stage-specific, or inducible promoter that is downstream from a second recombinase recognition sequence wherein stimulation of the promoter induces expression of the recombinase causing excision of DNA located between the recombinase recognition seqences.

26. (new) The method of claim 25 wherein the heterologous DNA is flanked 3' by two different recombinase recognition sequences or by repeats of the same recombinase recognition sequence.

27. (new) The method of claim 26 wherein the two different recombinase recognition sequences are LoxP and FRT.

28. (new) The method of claim 25 wherein the first recombinase recognition sequence is FRT.

29. (new) The method of claim 25 wherein the second recombinase recognition sequence is LoxP.

30. (new) The method of claim 25 wherein the recombinase encoding DNA expresses FLP or Cre.

31. (new) The method of claim 26 wherein the one recombinase encoding DNA expresses Cre and the other expresses FLP.

32. (new) The method of claim 25 wherein the gene cassette further comprises a marker gene located between the recombinase recognition sequences.

33. (new) The method of claim 32 wherein the marker gene is kanamycin resistance gene.

34. (new) The method of claim 25 wherein the heterologous DNA encodes a phenotypic plant trait.

35. (new) The method of claim 34 wherein the phenotypic plant trait is selected from the group consisting of growth habit, color, maturity, yield, mortality, sterility, disease resistance, metabolite production, and appearance.

36. (new) The method of claim 25 wherein the DNA encoding a recombinase expresses a protein is selected from the group consisting of FLP, Cre, R, Gin, PIV, FimB, C31, KW, SSV, IS1110/IS492, ParA and TnpX.

37. (new) The method of claim 25 wherein the organ-specific, developmental stage-specific or inducible promoter is selected from the group consisting of AG, AGL5, Bcp1, LAT52, PLENA, SIM, avrRp2 and alc.

38. (new) A gene cassette comprising:

a first nucleic acid recognition sequence for FLP;
a organ-specific, developmental stage-specific or conditionally inducible promoter for a gene encoding FLP;
a flp gene that encodes FLP;
a gene encoding a plant phenotypic trait; and
a second nucleic acid recognition sequence for FLP

wherein the nucleic acid recognition sequences for FLP are oriented as direct repeats.

39. (new) The gene cassette of claim 38 wherein the first or second recognition sequence for FLP is LoxP.

40. (new) The gene cassette of claim 38 wherein the first and second recognition sequences for FLP are LoxP.

41. (new) The gene cassette of claim 38 wherein the conditionally inducible promoter is a pollen specific promoter.

42. (new) The gene cassette of claim 41 wherein the pollen specific promoter is Bcp1 or LAT52.

43. (new) The gene cassette of claim 40 further comprising:

a first nucleic acid recognition sequence for Cre which flanks the first FLP recognition sequence;

a cre gene that encodes Cre positioned 5' or 3' to the gene encoding the plant phenotypic trait, said cre gene operably linked to a conditionally active promoter; and

a second nucleic acid recognition sequence for Cre which flanks the second FLP recognition sequence.

wherein recognition sequences are oriented as direct repeats.

44. (new) The gene cassette of claim 40 further comprising a marker gene positioned 5' or 3' to the gene encoding a plant phenotypic trait.

45. (new) The gene cassette of claim 40 or 43 wherein the promoter is selected from the group consisting of Pro-4 and AGL5.

46. (new) A gene cassette comprising:

a first nucleic acid excision sequence recognized by recombinase Cre;

a second nucleic acid excision sequence recognized by recombinase R;

a pollen specific conditionally active promoter operably linked to a nucleic acid that encodes FLP;

a third nucleic acid excision sequence recognized by recombinase FLP;

an externally activated promoter operably connected to a gene encoding recombinase FLP;

a transcription factor specific for the externally activated promoter;

a fourth nucleic acid recognition sequence recognized by recombinase FLP;

a fusion gene that encodes Cre and R and is linked to a protease sensitive site;

a DNA encoding a plant phenotypic trait;

a gene encoding a protease that cleaves the protease sensitive site between Cre and R in the expressed fusion protein;

a fifth nucleic acid excision sequence recognizable by recombinase R; and

a sixth nucleic acid excision sequence recognizable by recombinase Cre

wherein the nucleic acid recognition sites are oriented as direct repeats and substantially all DNA between the terminal excision sequence sites is deleted when the externally activated promoter is stimulated leaving no detectable DNA encoding the plant phenotypic trait and wherein the phenotypic trait is expressed only at the time of pollen maturation.

47. (new) The gene cassette of claim 46 further comprising a marker gene adjacent to the DNA encoding a phenotypic trait.

48. (new) The gene cassette of claim 46 wherein the protease sensitive site in the fusion gene is VRTQ/GPKR.